CLAIMS

1. A method for *in vitro* selection, from a library of catalyst molecules, of a catalyst molecule of interest having a relatively more efficient specific catalytic activity of interest as compared to the rest of the catalyst molecules within said library and wherein said *in vitro* selection method is characterised by that it allows multiple catalytic activity turn-overs (i.e. substrate to product catalytic activity turn-overs), by the catalyst molecule of interest, before it is finally collected and wherein said method comprises following steps,

(i) placing; a sample comprising a number of individual units wherein said sample comprises a library of catalyst molecules provided in the form of individual units, wherein the individual units comprise a first type individual unit having the following general structure:

C-S,

wherein C denotes a catalyst molecule and S a substrate which is capable of being catalysed into a product by at least one catalyst comprised within said library of catalyst molecules and thereby providing the possibility of obtaining a second type individual unit comprising the general structure:

C-P,

wherein C has the meaning defined above and P is the product molecule resulting from the catalytic conversion of the substrate S of the first type individual unit; and

(a) the substrate S is attached to the catalyst in a configuration that allows catalytic reaction between the catalyst and the substrate within said individual unit; and

(b) the nature of said attachment of the substrate and the catalyst provides the possibility, by means of a

characteristic of the product, of isolating an entity comprising information allowing the unambiguous identification of the catalyst molecule which has been capable of catalysing the reaction substrate molecule to product molecule;

under suitable conditions where a catalyst molecule of interest performs its catalytic activity of interest and where said method is characterised by that said sample is further under conditions wherein the product generated by a catalyst of interest is in contact with one or more reagent(s) which convert it back into the substrate S;

(ii) selecting for a catalyst of interest by selecting for one or more individual unit(s) which comprise(s) the product molecule; and

(iii) isolating an entity comprising information allowing the unambiguous identification of the catalyst molecule of interest which has been capable of catalysing multiple times the reaction substrate to product, by means of a characteristic of the product; and

optionally

(iv) repeating step (i) to (iii) one or more times by using the information comprised in said entity of step (iii) to generate the catalyst molecule of interest and construct an individual unit comprising said generated catalyst molecule of interest and then using this individual unit as a starting material in said repetition step.

2. The method according to claim 1, wherein the individual unit of point (i) in claim 1 is a biologically amplifiable individual unit.

3. The method according to claim 1, wherein the individual unit of point (i) in claim 1 is a biologically amplifiable individual unit and both said substrate and said catalyst molecule are attached on the surface of said biologically amplifiable individual unit.

4. The method according to any of the preceding claims, wherein said individual unit of point (i) comprises following structure: catalyst molecule - flexible linker - substrate.

5. The method according to any of the preceding claims, wherein said individual unit of point (i) in claim 1 comprises following structure: catalyst molecule - carrier system - substrate, or more preferably the structure: catalyst molecule - carrier system - flexible linker - substrate.

6. The method according to claim 2 and 4, wherein said carrier system of claim 4 within said biologically amplifiable individual unit of claim 2 is a phage.

7. The method according to claim 4, wherein said carrier system is a bead particle.

8. The method according to any of claims 1 to 7, wherein said library of catalyst molecules is a library of natural or unnatural peptides or polypeptides, preferably a library of enzymes.

9. The method according to claim 8, wherein said library is a library comprising polypeptides individually having a number of different enzymatic activities.

10. The method according to claim 8, wherein said library is a library comprising polypeptides variants derived from one or more precursor polypeptide(s), wherein said precursor polypeptide(s) exhibit(s) closely related enzymatic activities.

11. The method according to any of claims 8-10, wherein said library is a library comprising shuffled/recombined/doped polypeptides.

Sub a4

12. The method according to claims 1 to 7, wherein said library of catalyst molecules is a library of natural or unnatural nucleic acids.

13. The method according to claim 12, wherein said library is a library comprising nucleic acids having a number of different catalytic activities.

14. The method according to claim 12, wherein said library is a library comprising nucleic acid variants derived from one or more precursor nucleic acid(s), wherein said precursor nucleic acid(s) exhibit(s) closely related catalytic activities.

15. The method according to any of claims 12 to 14, wherein said library of nucleic acids is a library comprising shuffled/recombined/doped nucleic acids.

Sub a5

16. The method according to any of claims 1 to 7, wherein said library of catalyst molecules is a library comprising natural polymers molecules, or unnatural polymers molecules, or

small organic molecules, or small inorganic molecules or a mixture of said molecules.

17. The method according to claim 16, wherein said library is made by combinatorial chemistry.

18. The method according to any of the preceding claims, wherein the catalyst molecules and the substrate capable of being catalysed into a product (point (i) in claim 1) are of a different chemical substance.

19. The method for *in vitro* selection according to claim 1, wherein the catalyst molecules of interest are enzymes or proteins that have been coupled to an affinity tag, and wherein an optional step is performed prior to step (i) of claim 1, the optional step comprising an enrichment for individual units displaying (full length) enzyme or protein through a purification in which the units displaying the enzyme or protein are isolated by means of the affinity tag.

20. The method for *in vitro* selection according to claim 19, wherein the individual units displaying (full length) enzyme or protein are purified by the means of an anti-affinity-tag antibody column in which the units displaying the tagged enzyme or protein are isolated by means of the tag.

21. The method for *in vitro* selection according to claim 19, wherein the affinity tag comprises six histidine residues that are coupled to the C-terminal end of the enzyme or protein of interest, and the individual units displaying (full length) enzyme or protein are purified on a Ni-NTA column or on a anti-histidine antibody column, in which the

units displaying the tagged enzyme or protein are isolated by means of the tag.

22. The method for *in vitro* selection according to any of the preceding claims, wherein the selecting for a catalyst molecule of interest, in step (ii) of claim 1, is done by specific immobilization to said product molecule.

23. The method for *in vitro* selection according to any of the preceding claims, wherein the selecting for a catalyst molecule of interest, in step (ii) of claim 1, is done by the following strategy,

(i) constructing a system wherein substantially each of the individual units in step (i) of 1 comprising the substrate molecule and the catalytic molecule is bound to a matrix and wherein the unit is released from said matrix when the substrate is converted into the product; and

(ii) selecting for the unit(s) which are released from said matrix.

24. The method for *in vitro* selection according to any of the preceding claims, wherein the selecting for a catalyst molecule of interest (step (ii) of claim 1), is done by following strategy,

(a) constructing a product-column wherein a receptor specifically binding the product is placed along the matrix of the product-column; and

(b) adding the sample of individual units at one end of the product-column and selecting for the catalyst molecules of interest by isolating the individual unit(s) which arrive(s) latest to the opposite end on the column.

25. The method for *in vitro* selection according to claim 1, wherein the isolation of an entity comprising information which allows the unambiguous identification of the catalyst molecule of interest (step (iii) of claim 1), is done by physical or chemical procedures.

26. The method for *in vitro* selection according to claim 25, wherein the physical procedure is electrophoresis.

27. A method for producing a catalyst molecule of interest comprising performing the method for *in vitro* selection according to any of claims 1-18 and the further following step,

(a) producing said isolated catalyst molecule of interest in a suitable quantity of interest by a suitable production method.